eAppendix. Rationale for correcting blood lead for hematocrit or hemoglobin

More than 99% of whole blood lead exists in red blood cells. Therefore, whole blood lead measurements can be very closely approximated as a multiplication of two distinct quantities, lead concentration in red blood cells (erythrocyte lead) and the volumetric proportion of red blood cells in whole blood (hematocrit). As a biomarker of lead exposure, the erythrocyte lead may be better than whole blood lead since the latter can change as a result of adverse effects of lead exposure. Anemia may also be induced by something other than lead toxicity, affecting whole blood lead levels. These relationships give rise to confounding when association between lead exposure and an outcome caused by anemia is assessed using whole blood lead.

Erythrocyte lead can be measured directly by separating red blood cells by centrifuge and measuring lead content in the separated red blood cells, but it is not measured in NHANES. As a quantity equivalent to directly measured erythrocyte lead, we used the hematocrit-corrected blood lead. Hemoglobin is an alternative "divider" as it is very closely correlated with hematocrit owing to small inter-individual variation in hemoglobin concentration in red blood cells. Conceptually, the use of hematocrit- or hemoglobin-corrected blood lead can be likened to the widely accepted practice of expressing serum concentration of lipophilic contaminants on a per serum lipid basis. A notable feature of these two corrected blood lead measures is that they are not negatively correlated with the "divider" used in their derivation as expected from their form of "whole blood lead divided by hematocrit (or hemoglobin)." Rather hematocrit-corrected blood and hemoglobin-corrected blood are slightly positively correlated with hematocrit and hemoglobin, respectively (eTable 1, Supplemental Digital Content 1). These relationships follow because whole blood lead is practically equal to erythrocyte lead times the true hematocrit value, and, in calculating hematocrit-corrected blood lead, hematocrit denominator cancels out the true

underlying hematocrit level in the numerator. A similar calculation occurs in calculating hemoglobin-corrected blood lead as hemoglobin and hematocrit are approximately proportionate to each other.

On the other hand, compared with hematocrit- or hemoglobin-corrected blood lead, whole blood lead showed much stronger positive associations with hematocrit and hemoglobin (eTable 1, Supplemental Digital Content 1). This is expected for the subjects in this study because they were surveyed in 1999-2010 thus presumably were mostly free of overt hematologic effects from high levels of lead exposure.

eTable 1. Correlation among blood lead measures, hematocrit, hemoglobin, and serum iron: NHANES 1999-2010

	log10(Whole blood lead)	log10(Hematocrit-corrected blood lead)	log10(Hemoglobin-corrected blood lead)	Whole blood lead	Hematocrit-corrected blood lead	Hemoglobin-corrected blood lead	Hematocrit	Hemoglobin	Serum iron
log10(Whole blood lead)	1.000								
log10(Hematocrit-corrected blood lead)	0.985	1.000							
log10(Hemoglobin-corrected blood lead)	0.984	0.999	1.000						
Whole blood lead	0.833	0.827	0.826	1.000					
Hematocrit-corrected blood lead	0.818	0.835	0.834	0.986	1.000				
Hemoglobin-corrected blood lead	0.815	0.833	0.833	0.985	0.999	1.000			
Hematocrit	0.220	0.055	0.045	0.147	0.019	0.010	1.000		
Hemoglobin	0.248	0.078	0.079	0.169	0.037	0.037	0.968	1.000	
Serum iron	0.098	0.039	0.030	0.078	0.030	0.023	0.384	0.349	1.000

eTable 2. Minimally Adjusted Relative Risk for Cardiovascular Mortality from models with up to two metal biomarker variables: NHANES 1999-2010 with mortality follow-up through 2011

Model [a]	Blood Lead	Blood Cadmium	Serum Iron [b]	C-Reactive Protein	Serum Calcium [b]	Bone Mineral Density [c]
Models with hematocrit-corrected blood lead						
a1	2.14 (1.48–2.80)	2.50 (1.62–3.38)	None	None	None	None
a2	2.53 (1.79–3.28)	None	$1*10^{-5}$	None	None	None
a3	2.62 (1.87–3.37)	None	None	1.67 (1.37–1.96)	None	None
a4	2.66 (1.86–3.45)	None	None	None	$3*10^{-4}$	None
a5[d]	2.75 (1.81–3.70)	None	None	None	None	0.70 (0.19–1.20)
Models with hemoglobin-corrected blood lead						
b1	2.19 (1.52–2.87)	2.48 (1.61–3.36)	None	None	None	None
b2	2.57 (1.81–3.33)	None	$2*10^{-5}$	None	None	None
b3	2.67 (1.90–3.43)	None	None	1.66 (1.37–1.96)	None	None
b4	2.71 (1.90–3.53)	None	None	None	$3*10^{-4}$	None
b5[d]	2.81 (1.86–3.77)	None	None	None	None	0.69 (0.19–1.19)
Models with whole blood lead						
c1	1.88 (1.30–2.45)	2.53 (1.64–3.42)	None	None	None	None
c2	2.43 (1.72–3.14)	None	$3*10^{-6}$	None	None	None
c3	2.39 (1.70–3.08)	None	None	1.69 (1.39–1.99)	None	None
c4	2.39 (1.67–3.12)	None	None	None	$2*10^{-4}$	None
c5[d]	2.56 (1.69–3.43)	None	None	None	None	0.66 (0.18–1.15)
Models with no blood lead variable						
1	None	2.89 (1.90–3.88)	None	None	None	None
2	None	None	$3*10^{-6}$	None	None	None
3	None	None	None	1.67 (1.38–1.97)	None	None
4	None	None	None	None	$4*10^{-4}$	None
5[d]	None	None	None	None	None	0.82 (0.23–1.41)

Relative Risk per 10-fold increase and its 95% CI presented unless otherwise indicated. Adjusted for sex, race/Hispanic origin, smoking, and alcohol consumption.

[a] Each model is given a code for easy reference in the text.

[b] Modeled using natural spline therefore no single summary hazard ratio can be presented. Instead *p*-values for the set of natural spline terms are presented.

[c] Relative Risk per unit (g/cm^2) increase and its 95% CI presented.

[d] Models 5, a5, v5, and c5 were fit to a subset of the analytic sample due to limited availability for multiply imputed bone mineral density data.

eFigure 1. Association between serum calcium and hematocrit-corrected blood lead adjusted for sex, race/Hispanic origin, education, smoking status, alcohol intake, blood cadmium, and serum calcium, and C-reactive protein.